A68 Differential Extraction Conditions and the Premature Lysis of Spermatozoa: Effects on DNA Mixture Quantification and Amplification

Catherine M. Hennekens, MS, 51 Linden Street, Apartment 12, Allston, MA 02134; and Catherine M. Grgicak, PhD, and Robin W. Cotton, PhD, Boston University School of Medicine, Biomedical Forensic Sciences, 72 East Concord Street, L1004, Boston, MA 02118*

After attending this presentation, attendees will observe the effect Proteinase K concentrations, SDS concentrations, incubation times, and temperatures have on differential extraction efficiencies and the premature lysis of spermatozoa. This will allow attendees to optimize a preferential extraction procedure that will minimize sperm and non-sperm DNA mixtures. The attendees will also be able to differentiate between the differences in DNA concentrations derived from the extraction procedure versus the qPCR methods. Discussions on how to correct for qPCR irreproducibility will be discussed.

This presentation will impact the forensic science community by clearly demonstrating which differential extraction procedure to use in the laboratory to optimize the chemical means of sperm and epithelial DNA single source recovery, helping the forensic analyst/researcher understand the errors introduced during qPCR, and showing the DNA analyst/laboratory how to minimize these errors.

Many biological samples deposited at and collected from crime scenes contain mixtures from two or more individuals. The elucidation of individual donors in mixed biological samples has traditionally been a problem with serological testing and remains an issue today. Determining (1) the total number of contributors; (2) whether all of the data are present; (3) the relative ratio of DNA from each contributor; and, (4) whether a known individual is included or excluded, continues to be one of the most difficult and time consuming areas of DNA testing and training.

However, if the sample contains a mixture of sperm and epithelial cells, the DNAs may be segregated during the extraction process by utilizing differential extraction. In most laboratories, this is performed by chemical means, via the exploitation of the varying stabilities of cell membranes. The success of this type of extraction is based on the fact that sperm cell heads contain protein disulfide bonds in their outer membranes, making them impervious to lysis by treatment with Proteinase K and surfactant. The first step usually entails incubation of the sample in the presence of Proteinase K and surfactant (i.e., Sodium Dodecyl Sulfate (SDS)) to lyse epithelial cells, while leaving sperm cells intact. Following epithelial cell lysis, intact sperm are pelleted by centrifugation, allowing the DNA from the epithelial cells to be removed in the supernatant or "non-sperm fraction." Once removed, a second cell lysis is employed to extract DNA from sperm cells. In addition to Proteinase K and SDS, dithiothreitol (DTT) is usually added to reduce disulfide bonds in the sperm head, allowing access to the sperm's DNA resulting in a "sperm fraction."

It has been previously reported that considerable sperm lysis occurs simultaneously with epithelial cell lysis in the absence of DTT.[1] If sperm cells are lysed concurrently with epithelial cells there are two ramifications. First, DNA originating from sperm may be lost to the non- sperm fraction resulting in a lower DNA yield in the sperm fraction. Second, the profile obtained from the non-sperm fraction may be a mixture of DNA. The goal of this research was to analyze the effect Proteinase K concentrations, SDS concentrations, incubation times and temperatures had on differential extraction efficiencies and the premature lysis of spermatozoa.

The effect was quantified using the Quantifilier® Duo DNA kit, whereby the concentrations of male and female DNA in the non-sperm- and sperm- fractions were compared. To accomplish this, reproducibility studies designed to evaluate error in forensic qPCR analysis by assessing its source were performed. Methods designed to minimize qPCR errors were utilized to ensure differences in extraction concentrations did not stem from qPCR deviations. Three qPCR external calibration methods were explored, where the method which uses a validated curve as the external calibrator, is recommended due to its ability to increase sample throughput, reproducibility and eliminate the need to quality check DNA stocks from manufacturers. Finally, all samples were amplified utilizing the Identifiler® PCR Amplification kit and male/female mixture ratios of both fractions were analyzed and compared to those derived from quantification.

Comparisons between expected and observed ratios illustrated the quantity of female DNA in the sperm fraction is substantially affected by the absence of Proteinase K. Additionally, there was no indication of simultaneous sperm and epithelial cell lysis in the absence of DTT at Proteinase K concentrations ranging from 10 – 300 µg/ml. All other conditions exhibited minimal variation in DNA concentration when measured by qPCR. Therefore, despite the various protocols used for the differential lysis of epithelial and sperm cell mixtures encountered in casework, the method is robust and successful at most conditions tested.

Reference:

[1] Norris, Jessica V., et al. "Expedited, Chemically Enhanced Sperm Cell Recovery from Cotton Swabs for Rape Kit Analysis." Journal of Forensic Sciences 52 (2007): 800-5.

 ** Presenting Author*

DNA, Extraction, qPCR Error

 ** Presenting Author*